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Zuzana Heřmanová

The role of astrocytes in the formation of brain edema

Úloha astrocytů při vzniku mozkového edému

Bachelor's thesis

Advisor: Ing. Miroslava Anděrová, CSc.

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Prohlašuji, že jsem bakalářskou práci na téma ‚The role of astrocytes in the formation of brain edema' vypracovala samostatně, pouze za použití uvedené literatury a s pomocí odborných konzultací mé školitelky. Dále prohlašuji, že jsem tento text nepoužila na získání žádného akademického titulu.

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Abstract

Brain edema is a cause of mortality accompanying number of pathologies such as ischemia, traumatic brain injury, tumors or liver and kidney failure. It is described as a process of osmotic and water flux alterations, which lead to cell volume changes and to an increase in intracranial pressure. Brain edema is usually classified into two types: vasogenic and cytotoxic. Development of vasogenic edema is connected to the blood brain barrier disruption. Water accumulates in the extracellular space and exerts pressure on the cellular compartments of the tissue. The cytotoxic type of edema is characterized by water accumulation within the cells. The process of cellular volume enlargement is termed cellular swelling. Cytotoxic swelling is usually connected to glial cells, namely astrocytes, as these cells represent a part of the blood brain barrier and thus they influence homeostasis inside the brain. Water flows across cytoplasmic membrane through a system of specialized channels – aquaporins. For the brain edema formation, aquaporin 4 is the most important. It is localized on astrocytic membranes and using aquaporin-null mice, it has been shown, that it participates in water clearance in physiological and pathological conditions. Since the water fluxes are passive, the driving force for edema formation is the ionic imbalance. Changed concentrations of Na^+ and K^+ mainly drive water inside the cells. Ions are transported through channels, such as Na^+ / K^+ / Cl^- cotransporters, TRPV4, inwardly rectifying K^+ channels or volume regulated anion channels. The problematic of brain edema is studied intensively, but most of the experiments were performed using cultured cells or rodent models. However, the most recent studies suggest that situation in human brain is far more complicated, than has been thought before.

Key words:

Water transport, potassium and glutamate clearance, aquaporins, ion channels, transporters

Abstrakt

Edém mozku je příčina úmrtí doprovázející patologie, jako je například ischemie, traumatické poranění, nádory mozku nebo selhání jater a ledvin. Je popisován jako proces osmotických změn a změn v proudění vody, které vedou ke zvětšování objemu buněk a ke zvýšení nitrolebečního tlaku. Edém mozku je obvykle rozdělován do dvou typů: vazogenní a cytotoxický. Rozvoj vazogenního edému mozku je spojen s poškozením hematoencefalické bariéry. Voda se akumuluje v mezibuněčném prostoru a stlačuje buňky. Cytotoxický typ otoku je charakteristický akumulací vody uvnitř buněk. Proces buněčných objemových změn spojených s edémem mozku se nazývá buněčný ‚swelling'. Cytotoxický ‚swelling' je obvykle spojován s gliovými buňkami, především astrocyty, jelikož tvoří součást hematoencefalické bariéry a tudíž ovlivňují homeostázu v celém mozku. Voda proudí skrz cytoplasmatickou membránu systémem specializovaných kanálů – aquaporinů. Pro vznik edému je nejdůležitější aquaporin 4. Nachází se na membránách u astrocytů a použitím myší se zablokovanou expresí tohoto proteinu se ukázalo, že se podílí na odstraňování vody z tkáně při fyziologických i patologických podmínkách. Jelikož proudění vody je pasivní, hnací silou pro vznik edému mozku je iontová nerovnováha. Změněné koncentrace především Na^+ a K^+ pohánějí vtok vody dovnitř do buněk. Ionty jsou transportovány různými iontovými kanály / transportéry, jako jsou například Na^+ / K^+ / Cl^- kotransportéry, TRPV4, dovnitř usměrněné K^+ kanály nebo objemově řízené aniontové kanály. Problematika mozkového edému je velmi intenzivně studována, avšak většina experimentů je prováděna na buněčných kulturách nebo myších či potkaních modelech. Nejnovější studie nicméně naznačují, že situace v prostředí lidského mozku je daleko komplikovanější, než se původně myslelo.

Klíčová slova:

transport vody, vychytávání draslíku a glutamátu, aquaporiny, iontové kanály, transportéry

Contents

1 Introduction

In the human central nervous system (CNS), two main cells types can be identified: neurons, with the ability to transfer specific signals, and glial cells. These are specialized cell types carrying out functions, such as creating myelin coats around axons (oligodendroglia), inflammation and phagocytosis (microglia) or metabolic and homeostatic support (astrocytes). Experiments have shown that in healthy brain the cells occupy about 80 per cent of its volume (Nicholson & Sykova 1998). The rest 20 per cent represents the extracellular space (ECS), which is filled with isotonic liquid filtrated from blood by the blood brain barrier (BBB).

The whole brain is closed in a rigid structure of cranium. The brain tissue and the cerebral fluids infill the whole space inside the skull and therefore any changes in water content in brain tissue can lead to detrimental brain damage. In my thesis, I will describe mechanisms participating in brain edema formation, with an emphasis on the role of astrocytes.

2 Brain edema

The pathology of brain edema is known from studies as far back as the neurology came into the spotlight, because edema is the main cause of death in many brain diseases and injuries. In the middle of the 19th century, brain edema was considered to be an intracellular phenomenon, because the existence of functional ECS was denied. Along with the knowledge about ECS and the progress in molecular and microscopic techniques, a new concept of cerebral edema considering ECS appeared (Klatzo 1994).

In general, brain edema is defined as a complex process comprising changes in osmotic and water fluxes, that lead to an increase in intracranial pressure and to intracellular and extracellular volume changes (Unterberg et al. 2004; Ransom & Blank 1975). Verbatim, Klatzo described cerebral edema as an abnormal accumulation of fluid within the brain parenchyma producing a volumetric enlargement of the brain tissue (Klatzo 1994; Go 1984). Brain edema formation can result from or can lead to changes in brain parameters, such as intracranial pressure or integrity of BBB. But these are accompanying phenomena and were probably not taken into consideration in this brief definition.

Cerebral edema can be localized or generalized. It is the matter of time, cause of the edema and the seriousness of the injury or neuropathology, that led to its forming (Ransom & Blank 1975). By the process of diffusion, edema affects the whole brain and spreads from the area of the primary lesion. If it is not discovered early, brain edema causes intracranial hypertension and generalized ischemia (Rabinstein 2006). It is pathology with a very high mortality. Even if the medical intervention is timely, the outcome of the endured edema is usually neuronal damage with different seriousness, depending on the underlying pathology or injury (Faragó et al. 2016). It is even possible that cells survive almost intact (Go 1987).

It is known that people tend to sort all knowledge into arranged categories and taxons and even the pathology of brain edema is not an exception. With the discovery of ECS and its ability to accumulate abnormal amount of fluid, two major types of edema were described: *cytotoxic*, with a pathological influx of fluid into the affected cells, and *vasogenic*, characterized primarily by the BBB breakdown (Klatzo 1994) (Fig.1). This division is being used ever since. However, it is just a man-made tool and should not be used literally. In the pathological states, which are characterized by brain edema formation, the types shade into each other and usually define phases of a disease. Observing just one of the types is very rare and improbable (Nag et al. 2009). The most common brain diseases and damages accompanied by brain edema will be discussed later in this chapter.

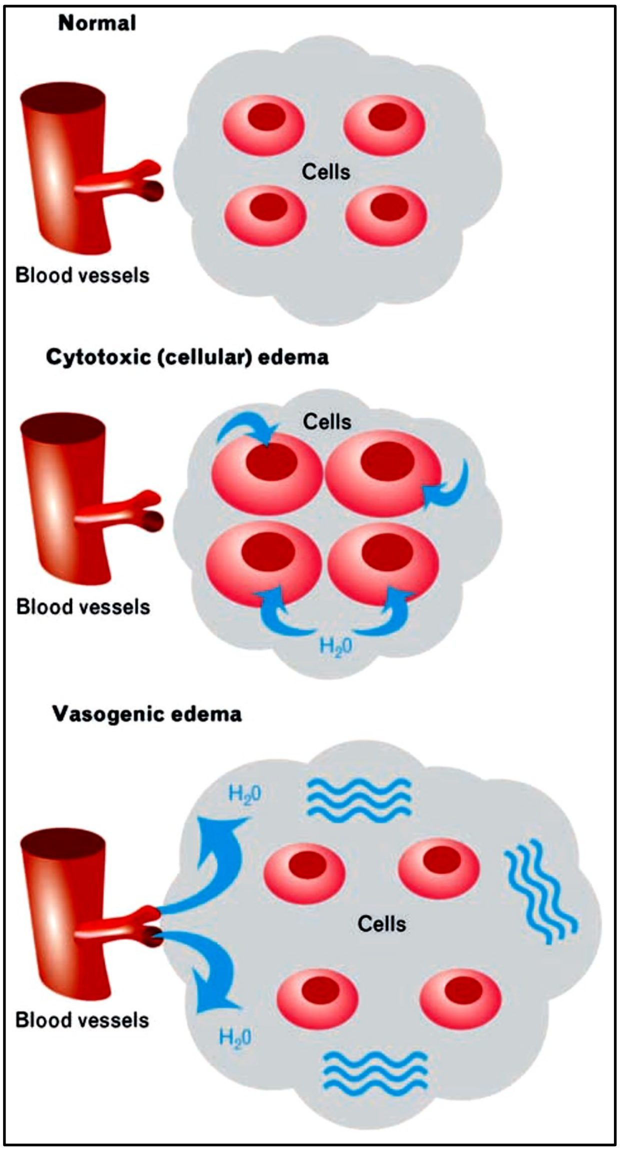

FIGURE 1: Schematic development of brain edema (Donkin & Vink 2010)

2.1 Vasogenic edema

Vasogenic edema is a type of brain edema, which is characterized by BBB damage. This barrier consists of complicated structures of capillary endothelium, pericytes and astrocytes with the main functions of maintaining brain homeostasis and selecting transported molecules. Vasogenic type of edema can be visualized post mortem by the use of antibodies against albumins, fibrin and other serum proteins (Nag et al. 2009; Nag 2003). In experimental animal models, usually specific tracers are used as the markers of the edema, for example Evans blue, which binds to serum albumins or horseradish peroxidase from plants. These molecules pervade brain parenchyma along with other blood proteins and thus demonstrate spreading of the edematous fluid through the defective BBB (Nag 2003). The mechanisms of BBB disruption differ depending on the present pathology. One option is a mechanical injury, which destroys vascular compartments, so the blood with its proteins can enter the ECS freely (Baethmann 1978). In clinical states without mechanical injury a significant increase in the number of endothelial vesicles, containing molecular tracers, was observed. So the process of transcytosis is considered to be the main route for passage of plasma proteins (Nag 2002).

The BBB disruption leads to an increase in permeability of capillary endothelium, mainly for proteins. When tight junctions between endothelial cells are damaged, a protein-rich plasma filtrate escapes from blood vessels into brain parenchyma (Unterberg et al. 2004). The efflux of edema fluid into ECS results in its enlargement. Vasogenic edema increases tissue water content, leading to the whole tissue swelling.

As discussed earlier, whole brain is closed in a rigid structure of the skull, which does not allow volume changes. Therefore any differences in intracranial volume are at risk for the brain tissue and can lead to its serious damage. It is obvious, that in case of brain edema, even the parts distant from the lesion are affected and are at risk of being damaged by the high pressure, to which they are exposed (Michinaga & Koyama 2015). However, the vasogenic edema does not spread with the same efficiency through gray matter as through the white matter. The preference of white matter is caused by a different vascular and cellular architecture. Unlike the gray matter, white matter is arranged more orderly. It consists mainly of parallel axons, which create long fibers. It has less cellular connections, so it is easier for the edematous fluid to overcome the forces holding cells together (Go 1984; Fishman 1975). Thanks to this, the white matter is more vulnerable to expansion of the edema liquid.

Spreading of this type of brain edema is determined by the systemic blood pressure, which influences the intravascular blood pressure in brain capillaries. This mechanism determines the force by which the edema fluid is being made - the higher blood pressure, the higher force of plasma filtrating (Go 1987). In the areas distant from the primary lesion, forming of edema is driven by the differences in osmotic pressures between plasma and the surrounding tissue. This imbalance forces the fluid to escape from blood vessels into ECS (Go 1987; Fenske & Prioleau 1978). Although water is eliminated through the glia limitans into subarachnoid space, through the undamaged capillaries, and via the ventricles into cerebro-spinal fluid, the amount of fluid escaping from capillaries is too high (Go 1987; Fenske & Prioleau 1978; Papadopoulos & Verkman 2007). The serum proteins from the ECS are degraded by glial uptake and digestion (Go 1987; Fenske & Prioleau 1978).

Due to the exposure to abnormal conditions, the swollen cellular elements are regularly found within the edematous brains. The changed environment of the brain tissue affects energy requirements of the cells and forces them to swell. Thus it is considered that vasogenic edema is in later phases accompanied by the development of a secondary cytotoxic type (Fig.2). The swelling of cellular compartments produces even larger decrease of ECS and aggravates already unfavourable conditions inside the edematous brain (Go 1987; Baethmann 1978).

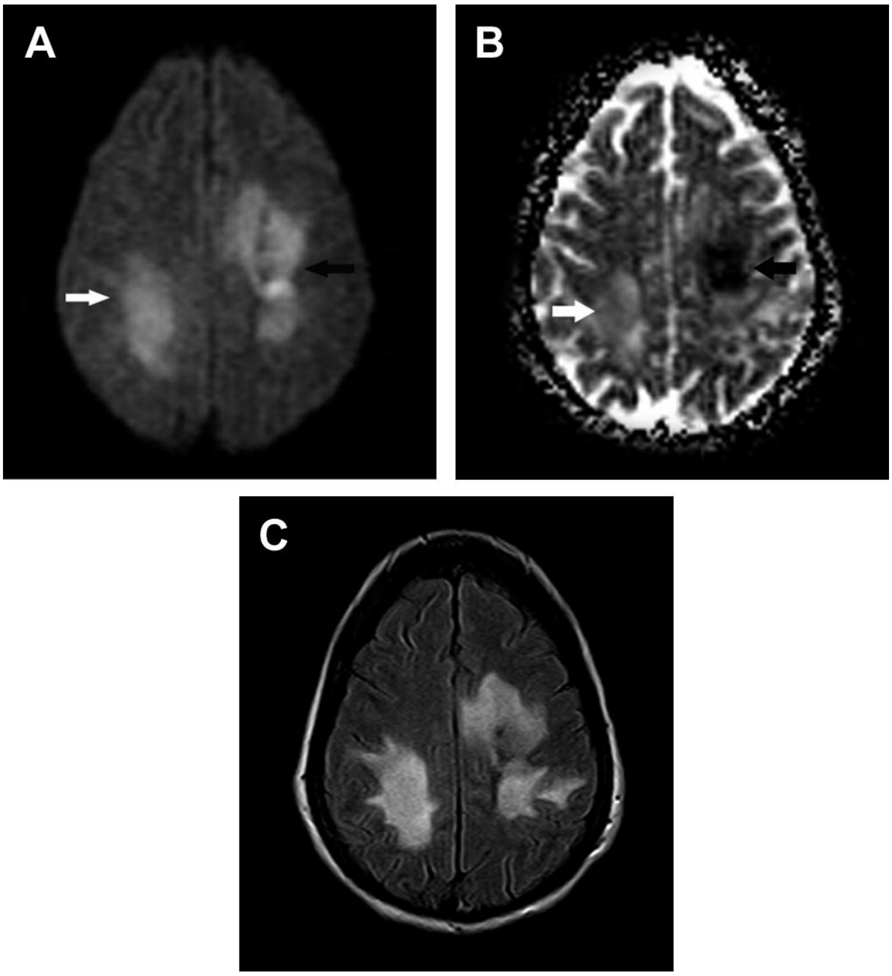

FIGURE 2: Magnetic resonance (MRI) scan showing a combination of cytotoxic and vasogenic edema. A, Diffusion-weighted sequence shows cytotoxic and vasogenic edema as bright signal; the bright signal corresponding to the area of vasogenic edema (white arrow) is due to “shine-through” effect from the T2 sequence. B, Apparent diffusion coefficient map clearly differentiates cytotoxic and vasogenic edema. Cytotoxic edema is associated with restriction in the movement of water molecules across the cellular membrane and thus with a low diffusion coefficient, which is seen as a dark signal (black arrow). Instead, vasogenic edema is associated with increased freedom of movement of water molecules, resulting in a high diffusion coefficient, which is seen as a bright signal (white arrow). C, Note that FLAIR sequence fails to distinguish between the 2 types of edema.

(Rabinstein 2006)

2.2 Cytotoxic edema

In contrast to vasogenic edema, in the cytotoxic type the ECS is reduced thanks to cellular swelling. It is a process of water and osmotically active solutes accumulation within the cells or cell compartments. Cytotoxic edema is essentially a water compartment shift with no change in tissue water content or volume. This occurs due to osmotic imbalance, when a tissue injury interferes with cellular osmoregulation (Ransom & Blank 1975). Another important difference is that in purely cytotoxic edema the BBB remains intact and any plasma filtrate does not escape into ECS (Go 1987; Baethmann 1978).

In laboratory conditions cytotoxic edema can be pharmacologically induced. Functioning of the pumping systems is blocked by inhibitors and ions enter the intracellular space (ICS) by an increased flow. These are not pumped out by transporting systems and therefore create significant osmotic imbalance leading to cellular swelling (Baethmann 1978).

2.2.1 Mechanisms of cellular swelling

Under physiological conditions, the uptake of osmotically active Na^+ and K^+ is compensated by their active elimination by Na^+/K^+ ATPase. But when this equilibrium is disrupted, the exchange pump is not able to follow increase in ion influx. The uptake of osmotically active solutes is substantially intensified by opening ion channels on synaptic membranes. All the ions entering the cells – Na^+ and Ca^{2+} mainly – are followed by molecules of water, which increase cellular volume – the cell swells (Klatzo 1994). The most significant in brain and the most examined by neuroscientists is an astrocytic swelling. Astrocytes play a key role in maintaining homeostasis in brain tissue and so when the concentrations of ions are altered, astrocytes respond to such conditions, which results in their volume changes.

However, astrocytes are not the only cells affected by the changed conditions within the brain tissue. After brain injury, neurons surrounding the lesion are activated above their physiological level. Even though the answer to it differs from the one of astrocytes, main cause of the neuronal swelling is the malfunction of Na^+/K^+ ATPase. In physiological conditions, the cell membrane is almost impermeable for Na^+ and the small amount of the molecules that reaches the ICS, is even more decreased by Na^+/K^+ ATPase. But when the pumping system is not functioning, the intracellular concentration of Na^+ grows. It soon reaches the threshold of excitation and causes a burst of action potentials. The ongoing influx of Na^+ and the action potentials end with a depolarization block of ion channels and thus neurons are not able to create another action potential. Using an *in silico* model it was discovered, that in about a day after a complete pumping system blockage the neuronal cells reach about 95 % of their final size. But in reality, the percentage of volume growth is lesser, because the

Na^+/K^+ ATPase functioning can be just lessen and not stopped completely, or the cell lysis before it reaches the maximal volume, which it achieved in model situations (Dijkstra et al. 2016).

Neuronal damage is connected to the functionality and morphology of oligodendrocytes, which create myelin sheaths. In pathological conditions, swollen compartments were observed in these glial cells – mitochondria, endoplasmic reticulum and Golgi complex. The edematous conditions in brain results in fragmentation of oligodendroglial processes and thus in neuronal demyelination, which increases neuronal damage (Castejón & Castejo 2015). Neuronal activation causes an increase in efflux of neurotransmitters – glutamate mainly – and leads to opening of ion channels on postsynaptic membranes and activation of transporting systems, that participate in neurotransmitter reuptake and metabolism. These systems are localized of course on neuronal membranes, but part of it is also integral to astrocytic metabolism.

2.2.2 Metabolic changes

Swelling of the cells is a typical feature of a developing cytotoxic edema. Transporting mechanisms dependent on adenosine triphosphate (ATP) are stopped, because of energy depletion. It is caused by mitochondrial impairment and lack of ATP. For example during ischemia, the lack of ATP results from an insufficient supply by glucose and oxygen. The glucose as a substrate for glycolysis, Krebs cycle and oxidative phosphorylation is an essential source of ATP. The lack of ATP as a universal cell energy storage molecule produces a slowdown of transporting processes.

Without oxygen supply, only anaerobic part of glycolysis is in motion and lactate is being synthesized along with fatty acids. This decreases cellular pH value and creates intracellular acidosis. Also the level of intracellular Ca^+ builds up because it is not pumped out of the cell, which leads to constant activation of a different set of genes. Under physiological conditions, the transcription of these genes is strictly regulated. Thanks to the mitochondrial malfunction, reactive oxygen species are synthesized in an increased extent and altogether, these changes lead to membrane disruption and irreversible damage and a necrotic center forms within the tissue (Rosenberg 1999). The osmotic changes are reversible at the beginning, but usually the original cause of the homeostasis disruption cannot be eliminated within few minutes, so it leads to the permanent damage (Nag et al. 2009).

2.3 Main pathologies accompanied by cerebral edema

As was mentioned earlier, brain edema is a life-threatening complication in numerous cerebral pathologies. One of the most common causes of death in industrially advanced countries is ischemic stroke. *Ischemia* – an insufficient blood perfusion – creates a metabolic imbalance. It can be caused for example by thrombosis, embolism, or by a compression of an artery. Primarily, ischemia results in cytotoxic type of edema because the affected tissue suffers from hypoxia and the glucose deficiency (Rosenberg 1999). The cytotoxic swelling occurs within few minutes after arterial obstruction. In the case of a permanent occlusion of a major artery, the energy deficiency causes a damage of the BBB and a secondary vasogenic edema develops thanks to the influx of serum proteins into the ECS (Go 1984). The risk of development of a cerebrovascular incident is increased by factors such as high blood pressure, smoking, obesity or diabetes mellitus.

Traumatic brain injuries (TBI) are a complex and heterogeneous group of brain disorders with the posttraumatic development of the brain edema. Laboratory simulations of the TBIs with as many accompanying changes and conditions as possible showed that TBI results in both vasogenic and cytotoxic type of edema. In the short time period after the injury, BBB is open for serum proteins and a vasogenic edema develops, but in a few hours, the BBB permeability changes and it remains open for small proteins only. The cytotoxic part occurs within a day or two as more cells become affected by the osmotic changes. The influx of ions into the cells drives more ions from the vasculature, which extends the edema (Donkin & Vink 2010).

Brain edema occurs as a complication and a possible cause of death also in acute liver failure. *Hepatic encephalopathy* – the neurological disorder resulting from liver failure – is a state with increased cerebral blood flow, elevated level of ammonia and increased intracranial pressure. It occurs after liver cirrhosis or during liver necrosis, which can be caused by hepatitis or other hepatotoxins. During this state, swollen astrocytes were observed as a result of increased level of ammonia. In the brain tissue, astrocytes are the main type of cells, which participate on ammonia removal, since it is used mostly for glutamine synthesis and the enzyme glutamine synthetase is localized in astrocytes only (Jayakumar et al. 2009; Butterworth 2015).

Among aforementioned pathologies, brain edema is a leading cause of mortality in patients with *brain tumors*. All the aggressive tumors create a secondary brain edema, no matter what cell type are they derived from (Murayi & Chittiboina 2016; Papadopoulos et al. 2004). Tumors themselves change tissue volume in the affected area, but producing also a secondary edema, the whole brain is at high risk of suffering an ischemic stroke. Tumorous tissue produces abnormal molecules, which affect cerebral vasculature and increase the BBB permeability (Papadopoulos et al. 2004). That allows increased entry of water into the brain parenchyma and extends the edema created by abnormal amount of fluid produced by the tumorous tissue. The analogous processes of brain edema formation to those described in this paragraph can appear also in connection with infections or hemorrhage (Nag et al. 2009; Papadopoulos et al. 2004).

3 Astrocytes

In the previous chapter, the cellular swelling was described as a typical feature of developing cytotoxic edema that affects both neurons and glia. Astrocytes are the most numerous population of glial cells and they outnumber neurons by a ratio of 1,4 : 1 in human brain (in rat brain the ratio is 1 : 3 for neurons). They are spread throughout the whole volume of brain, there is no region without some type of astrocytes (Sofroniew & Vinters 2010). Astrocytes have a specialized and very complicated morphology. Their soma is oval and relatively small, just 10 - 20 μm in diameter. From the soma a complex system of processes protrudes, some of them with widenings at the ends, called endfeet. These widenings surround blood vessels and brain surface. The processes are about 30 – 100 μm long, and are used for communication, transport of nutrients or for arranging contacts between capillaries and neurons. Thanks to these processes and gap junctions between them, astrocytes form a complex network through the whole brain (Sofroniew & Vinters 2010).

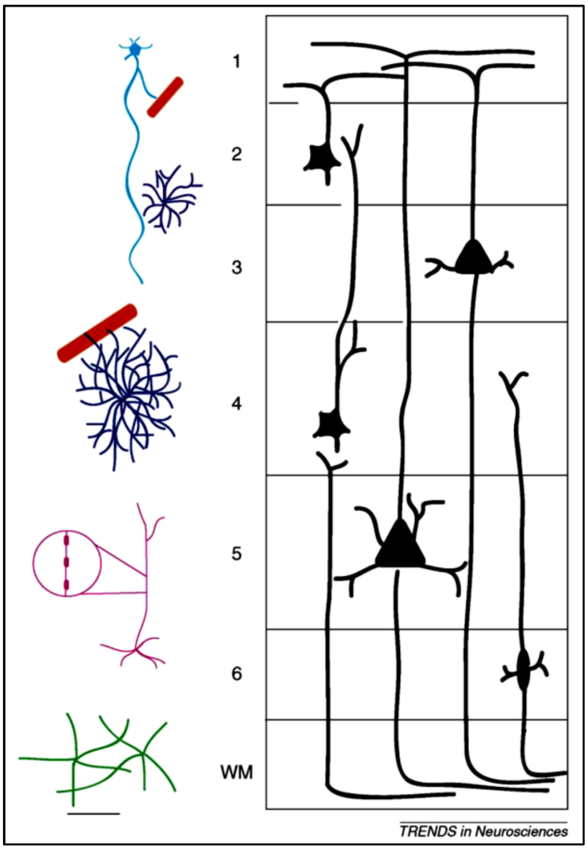

FIGURE 3: Distinct classes of human astrocytes (left) are located within different layers of the cortex (right). Primate-specific interlaminar astrocytes (light blue), protoplasmic astrocytes (dark blue) and blood vessels (red), polarized astrocytes (pink), fibrous astrocytes (green). Scale bar, 100 μm.

(Oberheim et al. 2006)

3.1 Types of astrocytes

On the basis of morphology and distribution, 4 types of astrocytes were described – *protoplasmic*, *interlaminar*, *polarized* and *fibrous* (Oberheim et al. 2006). The protoplasmic astrocytes are localized mainly in gray matter in deeper layers of the cortex, have irregular contours and their processes seem to fill most of the space around the soma and between other brain elements. The fibrous astrocytes are localized only in white matter and their morphology is more similar to neurons or fibroblasts. Both types of astrocytes (protoplasmic and fibrous) are present in rodent brain and can be also identified using tetanus toxin and monoclonal antibody A2B5 against specific polysialogangliosides (Raff et al. 1983), as demonstrated mostly on cultured astrocytes. Those cells, that were labeled by both these ligands, were determined to be fibrous astrocytes and those that did not bind any ligands, were termed protoplasmic cells (Raff et al. 1983; Miller & Raff 1984).

However, in human and primate brains, two more types of distinct morphologies of astrocytes were described. Interlaminar astrocytes are localized in the layer 1 of the cortex and have two types of processes. The long, usually unbranched type, that penetrate cortex and terminate in 3rd or 4th layer, and another type of processes that are short and are oriented to the pia mater and the brain surface. In the deep layers of the cortex near white matter, the second type of primate-specific astrocytes is localized - polarized astrocytes. They are unipolar cells with two long processes extended away from the white matter and their morphology resembles neurons. Both types (interlaminar and polarized astrocytes) can eventually extend processes to vasculature, but are usually minimally branched (Oberheim et al. 2006) (Fig.3).

3.2 Blood brain barrier and blood flow regulation

The integrity of the BBB is one of the features on the basis of which brain edema types are classified. Astrocytes are part of BBB and are considered to release signals for endothelial tissue to form tight junctions within brain (Janzer & Raff 1987). Thanks to this, the capillaries are impermeable and the only way for nutrients to get to neurons is through the endfeet of astrocytes which enclose brain vascular system (Fig.4). The astrocyte processes also surround neuronal synapses and are a part of neurotransmitter metabolism. However, creating BBB is not the only way how astrocytes can affect blood flow in the brain. Specialized mechanisms link neuronal activity with the regulation of cerebral blood flow, with astrocytes as an interface. The most important substances in those mechanisms are K^+ ions, the concentration of which is changing thanks to neuronal activity – for a review see Fröhlich et al. 2008. These changes influence endothelial cells (which are extremely sensitive to the K^+) and cause capillary dilatation. The whole process results in increase in blood flow at the regions of brain that are active at the moment, which means increase in oxygen and nutrients supply (Paulson & Newman 1987).

On the other hand, in case of brain edema and cellular swelling, the close coupling between astrocytes and capillary endothelium appears as a disadvantage. When the homeostasis is disrupted and the concentrations of ions do not correspond with physiological state, astrocytes increase their volume – they swell. These changes may result in compression of local capillaries and thus in interruption of local blood flow. This obstructions generate episodes of anoxia and ischemia and enlarge already existing brain edema (Pasantes-morales et al. 2000).

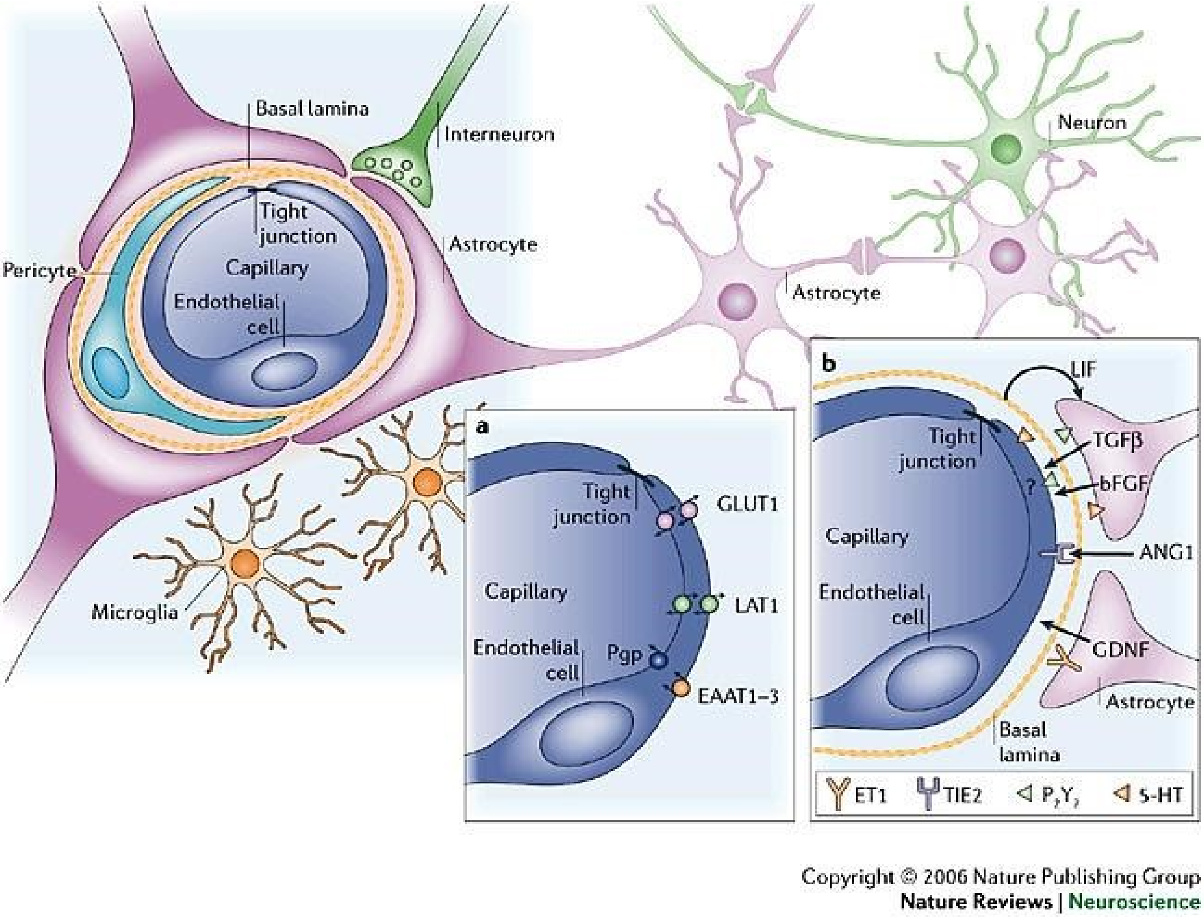

FIGURE 4: The barrier is formed by capillary endothelial cells, surrounded by basal lamina and astrocytic perivascular endfeet. Astrocytes provide the cellular link to the neurons. The figure also shows pericytes and microglial cells.

(Abbott et al. 2006)

3.3 Astrocytes in brain pathologies

For all neuropathological diseases (brain edema included) one feature is characteristic: inability of the tissue to maintain homeostasis. Since astrocytes are the main component of homeostatic processes, their role has been documented in many brain disorders. After head injury and CNS trauma or neurodegenerative diseases, a process of reactive astrogliosis appears. Astrocytes are capable of saving the integrity of the tissue by migrating to the area of lesion, transforming into reactive astrocytes and creating a glial scar. The process includes changes in gene expression and astrocytic hypertrophy. The activated reactive astrocytes produce cytokines – the molecules with pro- or anti-inflammatory effect – which modulate migration of other cell types, leukocytes mainly. They also help to restore fluid fluxes to the physiological level. The same process is also important during

CNS infections, because activated astrocytes are able to respond to inflammatory signals and also create them, thus they lead immune cells to the places of need. This reaction was observed in both bacterial and viral infections (Sofroniew & Vinters 2010).

On the other hand, reactive astrocytes cause neuronal degeneration, because they surround injured tissue. The loss of growth factors and nutrients delivery via astrocytes is a major obstacle for the regeneration of damaged axons (Ridet et al. 1997; Sofroniew 2005; Bush et al. 1999).

3.4 Mechanisms of volume changes in astrocytes

It was mentioned above that astrocytes are able to restore fluid fluxes to the physiological level. This holds true only in some cases, because for example in the pathology of brain edema, the amount of water entering the cells is too large to be reduced easily. However, dependent on water and ion distribution between ICS and ECS, cells are able to regulate their volume.

The most of intracellular water is bound to proteins or create solvation shells around ions, which means, there is not much space for free water molecules. The excessive influx of water leads to cellular swelling – the case of cerebral edema. On the other hand, loss of water – dehydration – results in cellular shrinkage and damage of protein structure, which results in the loss of their function. It applies also to the complexity and stability of plasmatic membrane or cellular cytoskeleton. Water is able to permeate the plasma membrane easily due to the presence of aquaporin (AQP) channels and its movement is driven by osmotic pressure (Lang 2007; Law 1994).

Astrocytes possess the ability to control their volume and change it in response to different intra- and extracellular conditions. The volume regulation is required even if the cell is in perfectly isotonic environment. Thanks to transporting processes and metabolism, the level of intracellular water changes and needs to be regulated. Otherwise volume changes can have dramatic consequences because of the rigid structure of cranium. The constant shape of every cell is being kept and regulated by cytoskeleton. Therefore it is obvious that every change in cellular volume must affect the system of cytoskeleton filaments. Their disruption and reorganization is reflected on ion channels and plasma membrane transporting systems, which are associated with them. The cytoskeleton is also essential for vesicle-mediated transport and integrating transporters into plasmatic membrane on the surface of the cells (Pedersen et al. 2001). The cells can recover to the normal volume by modifying osmotic gradients. This requires volume sensors, which can activate signaling cascade and lead to changes in transport of osmotically active solutes – both organic and inorganic. Once the volume sensors recognize the cell volume is back on physiological level, the changes are stopped. The process of volume recovery is termed *regulatory volume decrease* (RVD) or *regulatory volume increase* (RVI) (Lang 2007; Pasantes-Morales et al. 2000) (Fig.5).

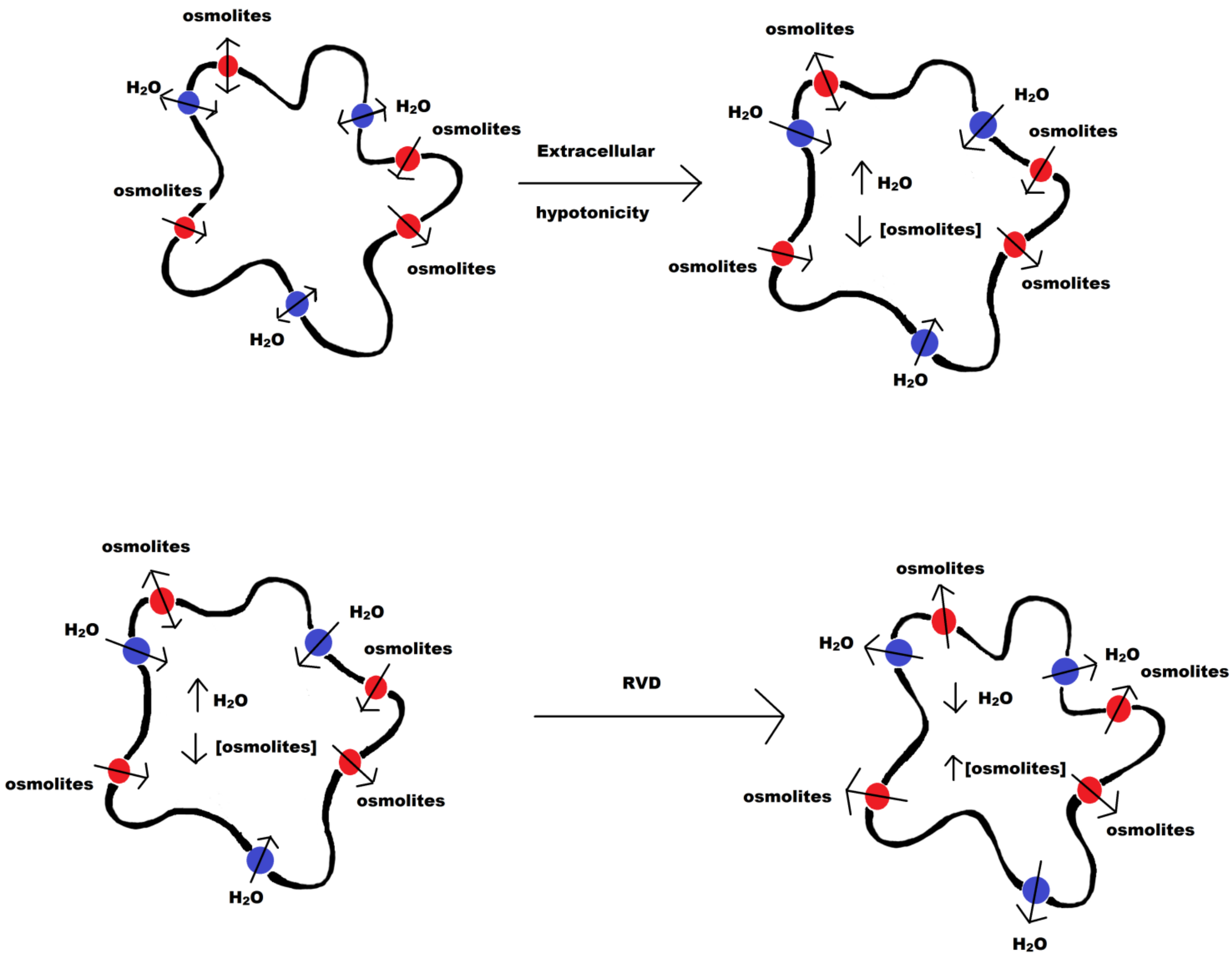

FIGURE 5: Mechanism of RVD. Extracellular hypotonicity causes water influx into the astrocytes (upper part), which activates volume sensors. Cells start to efflux osmotically active solutes. These are followed by passive efflux of water and the cellular volume recovers to the physiological level (lower part).

4 Channels and transporters

Maintaining homeostasis and volume changes require involvement of transporting systems and channels in plasmatic membranes of the astrocytes. The RVD and RVI are strictly regulated processes of ionic transport into the cells or to the ECS. This transport and changes in ionic concentrations drive passive water fluxes in brain. Specialized protein structures in the plasmatic membranes are needed for ions and even for the water to flow through the membranes. These channels are studied intensively as a possible target for anti-edema drugs, which can lessen negative impact of brain edema.

4.1 Aquaporins

Cellular volume, as all the functions of all cell types, depends on water. Water creates environment in the cells and also in ECS. Human bodies consists of about 70 % water and also other groups of organisms are composed mainly of water (Agre 2004). More than 90 % of molecules in the body are water and all transport processes depend on it (Amiry-Moghaddam & Ottersen 2003). Water can diffuse into cells passively through the plasmatic membrane, but this process is insufficient. This type of water influx is quite slow, bidirectional and cells do not have any regulatory mechanisms to control it (Amiry-Moghaddam & Ottersen 2003). However, simple diffusion is not the only mechanism available. In 1990s, the first member of AQP channel family was identified by Agre et al. (Nag 2003; Preston et al. 1993; Agre & Kozono 2003; Agre et al. 2002). But the existence of mechanism that allows water to permeate membranes easily was suggested long before (Agre 2004). To date, 11 AQPs are described in mammals and more than 300 in lower organisms (Amiry-Moghaddam & Ottersen 2003; Tait et al. 2008). AQPs allow water transport in the direction of greater osmolality. They are thought to be activated permanently, because no gating or regulatory mechanisms were identified, so water flows passively through the channels and follows osmotic gradients (Agre et al. 1993).

AQPs are small (about 30 kDa) transmembrane protein channels usually functioning as tetramers. Each monomer contains separate and independent pore. Moreover, the tetramer together creates another pore in the center of the structure (Fukuda & Badaut 2012; Badaut et al. 2013). The selectivity of every pore is determined by steric and electrostatic factors (Papadopoulos & Verkman 2013). Nevertheless, water molecules are not the only ones that can pass through the channels. Two subgroups are being described. The first subgroup includes AQP0, 1, 2, 4, 5, 6 and 8. They are primarily permeable for water only, but through the central pore also O_2 or CO_2 can pass. Yet, this process was shown only in few types of AQPs (AQP1, 4 and 5). The second subgroup is named aquaglyceroporins, including AQP3, 7, 9 and 10. These channels are permeable not only for water, but also for glycerol, urea and some small polar solutes (Tait et al. 2008; Papadopoulos & Verkman 2013; Badaut et al. 2013).

4.1.2 Types of aquaporins in brain

In the brain, mainly two subtypes of AQP channels can be found. The first is AQP1, which is localized in choroid plexus and plays an important role in secretion of cerebrospinal fluid (Agre et al. 2002). The second is AQP4. This protein is localized on astrocytic endfeet, that surround capillaries and helps to create BBB, and on the endfeet of glia limitans. This type was also found on the membranes of ependymal cells (Rash et al. 1998) (Fig.6). AQP4 has many specifics. It exists in two

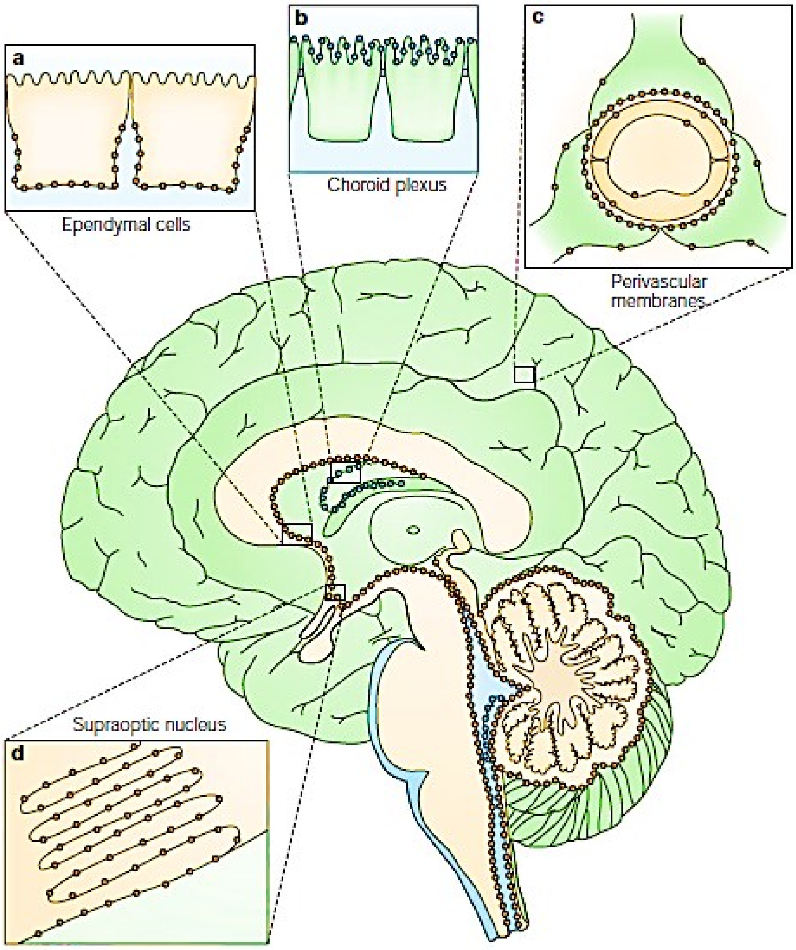

FIGURE 6: AQPs in the brain. Distribution in brain of AQP1 (blue) and AQP4 (orange) schematically illustrated on a sagittal section of a human brain.

(Amiry-Moghaddam & Ottersen 2003)

isoforms - M1 and M23 – each with different N-terminus, because of different translation initiation. The translation can start at two methionine codons – the first one at N-terminus, when the product is 323 amino acids long, or another one at the position 23. The protein is than 301 amino acids long. These isoforms create heterotetrameric channels. However, it was demonstrated, that the M23 form is expressed more often. The ratio between both isoforms is 3 : 1 (Neely et al. 1999). It was also demonstrated that the water permeability of M23 isoform is lower, than the permeability of M1 (Fenton et al. 2010). The expression of AQP4 in astrocytes is so heavy, that in freeze fracture studies, AQPs form microcrystals known as square arrays (Rash et al. 2004). The size of every crystal and their number is determined by the composition of every tetramer of AQP and by the ratio of both isoforms (Rash et al. 2004; Furman et al. 2003). In the brain tissue, also AQP9 is being expressed. This channel is a member of aquaglyceroporin subgroup. It was reported to be present on endothelial cells, glia and neurons. It probably plays a role in energy homeostasis (Badaut & Regli 2004; Arciénega et al. 2010).

4.1.3 Aquaporins and brain edema

Brain tissue is probably the most sensitive part of the body to osmotic changes. Even small differences in concentrations of solutes or in water content can lead to development of a pathological state. Since the AQP4 is the most common type of water channel inside the brain tissue (Papadopoulos & Verkman 2013), its effect in brain pathologies is studied very intensively. It is supposed to play a key role in development of cytotoxic cerebral edema. This evidence came from experiments with AQP4-null mice. In a model of water intoxication, when every mouse was given an intraperitoneal water injection, wild type mice showed brain swelling and neuronal damage linked to it. These mice had also very high mortality range. On the other hand, AQP4-null mice remained just mildly affected and most of them survived with less significant impact (Manley et al. 2000). Another model of cytotoxic brain edema is bacterial meningitis. By this infection, brain swelling is the major neurological complication. Injection of a suspension of *Streptococcus pneumoniae* was given to two mice populations, wild type and the AQP4-null mice. Both showed neurological impairment, but the population of AQP4-null mice had significantly lower mortality and damage. Also intracranial pressure was lesser in AQP4-null mice (Papadopoulos & Verkman 2005). In the case of using middle cerebral artery occlusion (MCAO) model, AQP4-null mice showed significant reduction of infarcted area, compared to the wild type mice. Also the tissue swelling was decreased in AQP4-null mice. These results are related to the improved neurological outcome of AQP-null mice (Yao et al. 2015).

Increase in water content during brain edema results in upregulation of AQP4 expression. It was shown in several culture and animal models (Papadopoulos & Verkman 2005; Tait et al. 2010; Ke et al. 2001; Vizuete et al. 1999). The upregulation can be caused by effort of astrocytes to increase efficiency of the water transport through BBB and into capillaries. It was shown, that in vasogenic edema, water is eliminated through the glia limitans and into subarachnoid space and AQP4 functions as a passage way (Papadopoulos & Verkman 2007). In AQP4-null mice, the vasogenic edema is increased thanks to the lack of available passages. The water accumulates inside the brain parenchyma and worsens spreading of edema (Vindedal et al. 2016). In cytotoxic edema, dying cells release their content into ECS. The increased concentration of intracellular ions causes passive efflux of water from surrounding astrocytes into the ECS. It is suggested, that water elimination in this stadium can be similar to that in vasogenic edema (Papadopoulos & Verkman 2007). But the increased number of AQP4 causes increase in BBB permeability, thus more water is entering the brain tissue from capillaries. It is a kind of a vicious circle: more AQPs, more water; more water, more AQPs. In AQP4-null mice the cytotoxic swelling is decreased, because water is not able to escape from the blood vessels into brain parenchyma (Vella et al. 2015).

4.2 Na^+ / K^+ / Cl^- cotransporter (NKCC)

Passive water influx to the astrocytes is driven by osmotic gradients created by unequal ionic concentration on both sides of plasmatic membranes. A group of membrane proteins that are substantial for maintaining of intracellular ionic concentrations are Na^+, K^+, Cl^- dependent cotransporters (NKCC). These cotransporters are electroneutral transporting systems, which transport ions of Na^+, K^+ and Cl^- (Fig.7). Two isoforms of the cotransporter have been described and named NKCC1 and NKCC2. The NKCC2 isoform was found only in the cells of kidney and so its function is not related to the pathology of brain edema. NKCC1 cotransporter is, however, localized on plasmatic membranes of many cell types including astrocytes, neurons and oligodendroglia (Su et al. 2001). It also has a significant role in the regulation of the cellular volume and it is supposed to participate in

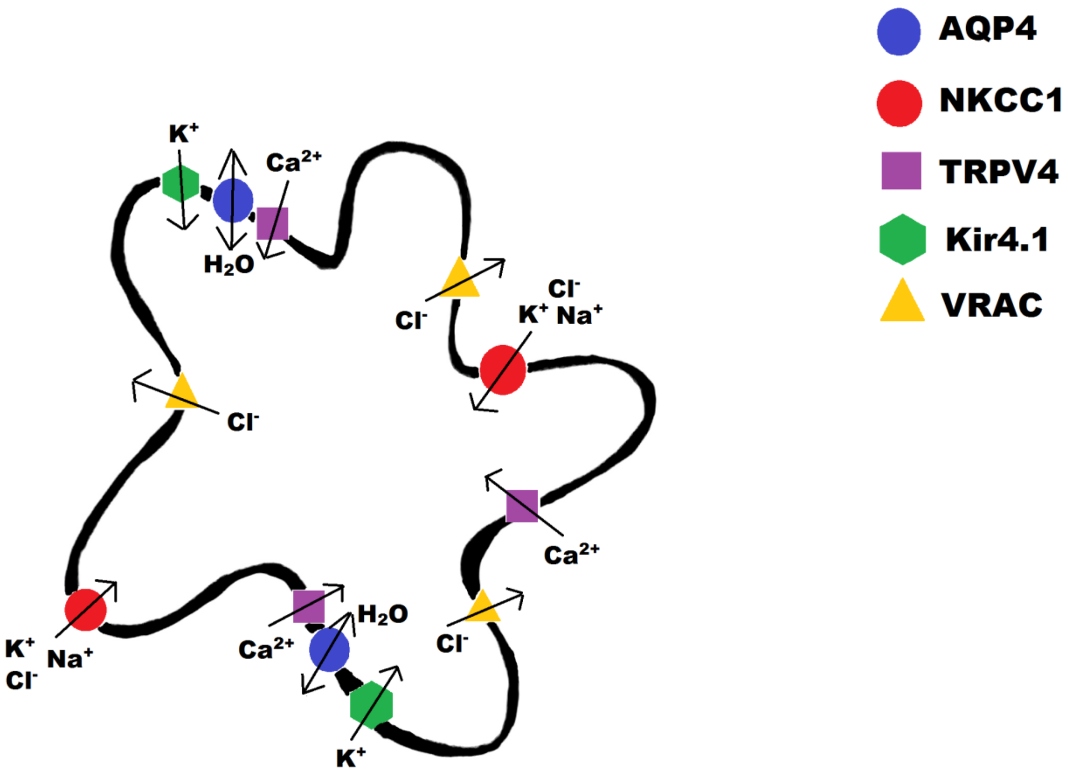

FIGURE 7: Direction of transport through AQP4, NKCC1, transient receptor potential channel – vanilloid type 4 (TRPV4), inwardly rectifying K^+ channels (Kir4.1) and volume regulated anion channel (VRAC) on astrocytic membrane. Figure shows just the main ions and molecules transported through the channels.

K^+ uptake by astrocytes. It was demonstrated, that increased extracellular concentration of K^+ activates NKCC1 (Yan et al. 2001; Lenart 2004).

NKCC1 transports ions into cells with the stoichiometry of Na^+: K^+: $2Cl^-$ (Jayakumar et al. 2012). The cotransporters seem to react to a various scale of stimuli. Number of studies has described activation of NKCC1 by oxidative stress in case of ischemia or TBI and its participation in response to astrocytic swelling (Lu et al. 2016). The expression and activity of NKCC1 is increased by hypoxia and glucose deprivation through an activation of kinases and phosphorylation of N-terminus (Yan et al. 2003).

The increased uptake of Na^+ results in expansion of cytotoxic edema, because increased intracellular concentration of Na^+ ($[Na^+]_i$) drives water through AQP4 channels to the ICS. In cultured astrocytes in simulated pathological conditions, development of cytotoxic swelling can be decreased by use of bumetanide (Badaut et al. 2013). This inhibitor decreases function of NKCC1 cotransporter and thus reduces cellular swelling, but only when the administration is pre-ischemic. In case of post-pathological administration, the protective effect was not observed (Yan et al. 2003). It was also described, that bumetanide is able to decrease AQP4 permeability for water. But since this molecule affects NKCC1 cotransporter primarily, the decrease in water passage can be due to decreased ionic concentrations inside astrocytes (Badaut et al. 2013). Another inhibitor is for example furosemide, which has an effect on water flow through AQP4 channels, as bumetanide does. The inhibition of both channels together decreases swelling of astrocytes and has neuroprotective effect (Su et al. 2001; Jayakumar et al. 2012; Migliati et al. 2010).

4.3 Potassium channels

The predominant function assigned to astrocytes is to buffer extracellular K^+. Potassium is released during neuronal excitation and its uptake via astrocytes is the primary mechanism of maintaining extracellular K^+ concentration ($[K^+]_e$) on physiological level. The elevated $[K^+]_e$ causes hyperexcitability and neuronal death and also can result in astrocytic swelling. Thanks to its crucial role in the brain tissue functioning, the astrocytic K^+ channels are studied intensively both in healthy brain and in the conditions of brain pathologies (Seifert et al. 2016). K^+ channels, NKCC1 together with Na^+/K^+ ATPase participate in K^+ clearance and induce astrocytic swelling.

Astrocytes – as other cell types – express many different K^+ channels. The channels transporting only K^+ can be divided into three main groups: voltage-gated K^+ (K_V) channels, inwardly rectifying K^+ (Kir) channels and two-pore domain K^+ (K_{2P}) channels (Ryoo & Park 2016).

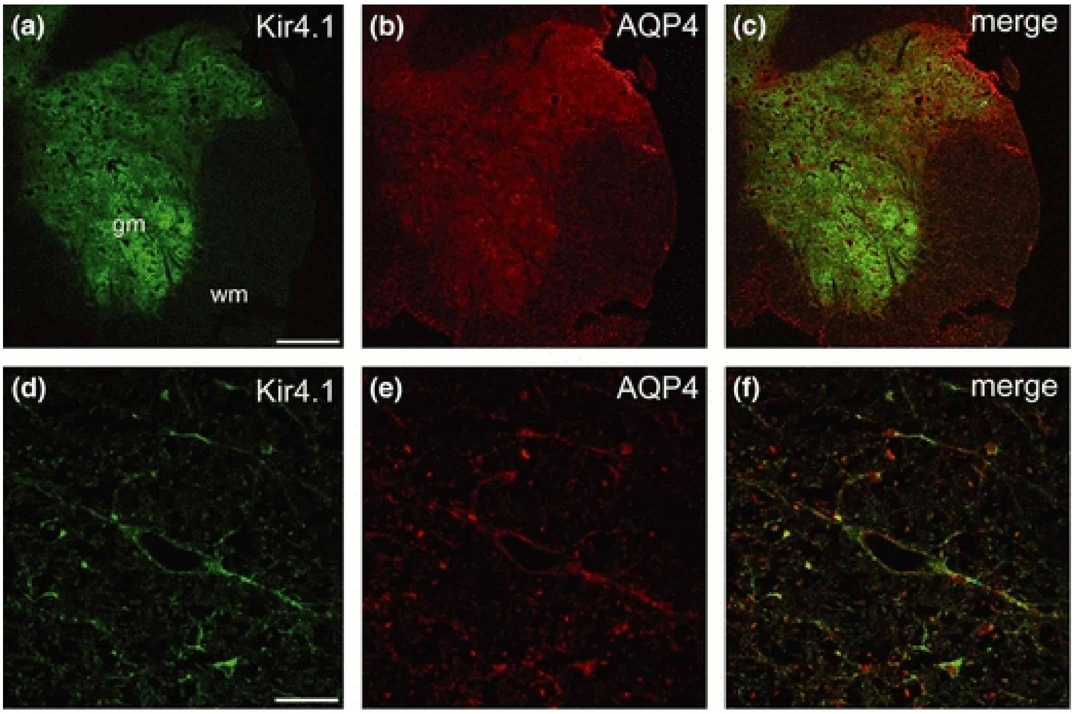

FIGURE 8: AQP4 is co-expressed with Kir4.1 in gray matter astrocytes. (a–c) Cross-sections from a P14 mouse, double-labeled with Kir4.1 (green) and AQP4 (red). Both proteins are predominantly observed in the gray matter while white matter areas are spared. (c) represents the overlay of (a) and (b) showing a marked overlap of AQP4 and Kir4.1 staining. (d–f) represents high resolution images of an area from the gray matter (taken from above section). AQP4 and Kir4.1 are co-expressed predominantly on perivascular domains. (f) Represents the overlay of (d) and (e). Bar: 50 µm (a–c) and 20 µm (d–f).

(Dibaj et al. 2007)

4.3.1 Kir4.1 channels

On the astrocytic membranes, Kir4.1 subtype of inwardly rectifying K^+ channels was identified, but it is not expressed by all astrocytes. Kir4.1 was not found in neurons. Its localization is mainly in membranes surrounding synapses and capillaries. Thanks to its localization in astrocytes, this channel is supposed to be part of K^+ buffering system. It is suggested that its expression on some astrocytic processes is a response to extracellular signals (Higashi et al. 2001). Kir4.1 is a channel with one pore domain. In the astrocytic endfeet, the Kir4.1 channels were found in close coupling with AQP4 (Fig.8). Experiments have shown that the presence of AQP4 adds volume sensitivity to the Kir4.1 and so it is able to respond to the changing water content and to cellular swelling in the case of brain edema (Seifert et al. 2016).

4.3.2 Two pore domain K^+ channels

One of the main astrocytic functions in brain tissue is K^+ buffering. Two pore domain K^+ channels (K_{2P}) permit K^+ transport through the plasmatic membranes. K_{2P} channels, such as TWIK-1 or TREK-1, are protein homodimers responding to various signals by creating an instant K^+ current, the direction of which depends on the protein subtype and on K^+ driving force. Activation of these channels can be caused by physical stimuli or by biological or chemical signaling. TWIK and TREK channels are suspected to be the main mediators of K^+ passive conductance in astrocytes. These channels are outwardly or inwardly rectifying and so they may be activated by pathological conditions and thus participate in spreading of brain edema (Ryoo & Park 2016; Zhou et al. 2009).

4.4 Transient receptor potential channel – vanilloid type 4 (TRPV4)

The above described complex of AQP4 and Kir4.1 channels is suspected to be accompanied by another non-selective cation channel, TRPV4, and all together they play a key role in regulation of astrocytic volume via transporting cations and water molecules (Jo et al. 2015).

TRPV4 is a member of a protein family of transient receptor potential (TRP) channels. These are tetrameric structures present in plasmatic membranes of a wide range of species and tissues and are usually described as polymodal receptors (Benfenati et al. 2007). Mammalian TRP channels are divided into six subfamilies and the letter V in TRPV subfamily stands for vanilloid (Shibasaki 2016). TRPV4 is able to respond to osmotic stress and it seems to be activated by cellular swelling (Benfenati et al. 2007). It was also shown, that – along with other functions – this type of channel is thermosensitive, and it also plays an important role in the process of nociception (Shibasaki 2016).

TRPV4 localization has been shown in astrocytic plasmatic membranes of the subpopulation of about 20 - 30 % of astrocytes (Shibasaki et al. 2014). Despite being a non-selective channel, it displays a weak Ca^{2+} selection, so it is supposed to be the key-player in the Ca^{2+} signaling of astrocytes. TRPV4 is able to increase intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by allowing the extracellular Ca^{2+} ions entering the cells (Benfenati et al. 2007). In pathological conditions of brain edema, TRPV4 channels are activated by astrocytic swelling and their activation causes an increase in the swelling, probably by activating phospholipase A2 an increasing $[Ca^{2+}]_i$ (Iuso & Križaj 2016). Its over-activation starts at about one hour after ischemia and reaches its maximum in about 7 days (Butenko et al. 2012). This is suggested to affect the activity of AQP4 and its permeability for water and thus to play a significant role in volume regulation (Ryskamp et al. 2014). The increase in $[Ca^{2+}]_i$ in astrocytes, however, results also in release of neurotransmitters, such as glutamate, from neuronal cells. It modulates synaptic activity and can lead to formation of a depolarization block (Shibasaki et al. 2014).

4.5 Volume regulated anion channels

VRACs – volume regulated anion channels – are another type of proteins activated in response to cellular swelling. These are heteromeric structures of LRRC8 protein and are suggested to create hexameric complexes (Syeda et al. 2016; Voss et al. 2014; Lutter et al. 2017). With at least five different genes for the subunits, the number of different subunit combinations of VRACs is quite high. It was discovered, that from these subunits the one named LRRC8A is essential for functioning of the channel and for its localization in plasmatic membranes (Fig.9). From the other subunits, at least one must be present in the VRAC complex (Voss et al. 2014). VRACs have a wide range of different functions (probably thanks to the changing subunits), depending on the cell type and conditions within the tissue. They are permeable not only for anions but also for small organic molecules (Lutter et al. 2017).

Activity of these channels is one of the main mechanisms of RVD and the efflux of Cl^- through VRACs together with release of K^+ via specialized channels helps to restore physiological volume of the astrocytes. Ions are followed by the molecules of water and the volume of an astrocyte decreases. VRACs thus provide a driving force for water efflux (Benfenati et al. 2011). But with the release of Cl^-, opened VRAC channels are permeable also for excitatory amino acids. These function as neurotransmitters and in pathological states, the increased level of glutamate is toxic for neurons (the process of glutamate excitotoxicity will be discussed later) (Lutter et al. 2017; Mongin 2015).

VRACs are dependent on the ATP supply. In case of ischemia, when the lack of ATP is one of the reasons for cellular swelling, these channels are not functioning and do not decrease cellular volume (Lutter et al. 2017; Mongin 2015). The lack of ATP also affects the Na^+/K^+ ATPase. Changed $[Na^+]_i$ induces negative response of the VRAC channels. The decrease of VRAC functioning occurs in

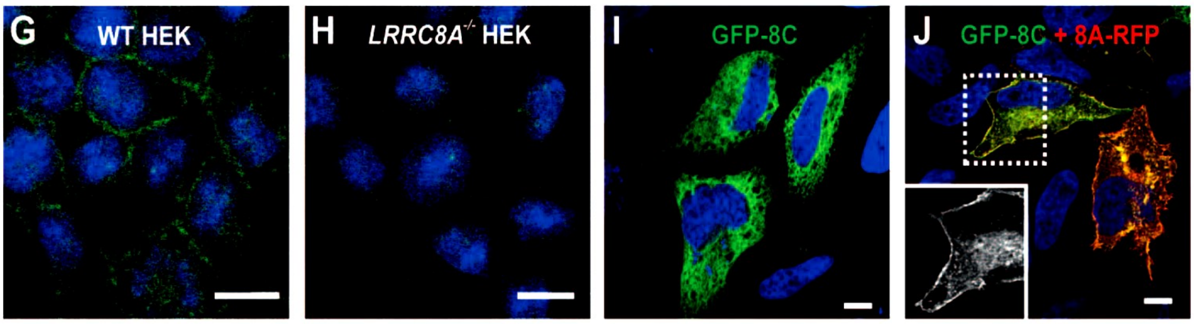

FIGURE 9: siRNA screen for volume-regulated anion channel VRAC identifies LRRC8A. (G) Plasma membrane localization of endogenous LRRC8A in HEK cells. (H) No LRRC8 labeling in LRRC8A−/− HEK cells. (I) LRRC8C is intracellular when transfected into HeLa cells, but (J) reaches the plasma membrane when cotransfected with LRRC8A. (Inset) Magnification of boxed area showing only GFP fluorescence. Scale bars, 10 mm.

(Voss et al. 2014; adjusted)

both intracellular hypertonicity and extracellular hypotonic conditions. This suggests, that VRAC channels are able to respond to changed ratio of $[Na^+]_i$ to $[Na^+]_e$, even if one of the parameters is not changed. The condition is that $[Na^+]_i$ appears increased (Minieri et al. 2015). However, it was still not discovered, whether VRACs do have any sensor in its own structure or do they need another cellular compartments to recognize non-physiological conditions (Syeda et al. 2016).

4.6. Glutamate

Brain edema is a dangerous state, because it leads to neuronal and astrocytic damage and to the cellular death eventually. In case of TBI or any other brain pathology, neuronal tissue is excited above physiological level. The main excitatory neurotransmitter in the CNS is glutamate. Its activity is associated with binding to its receptors on postsynaptic membranes. Glutamate uptake system is located on both astrocytic and neuronal membranes. Astrocytic processes surround the synapses completely and prevent glutamate from diffusing to another synapse (Hertz et al. 1999). The process of glutamate clearance is very fast and prevents the concentration of extracellular glutamate to reach toxic levels.

In the previous paragraph, VRAC channels were discussed. Besides from transporting anions, they are opened also for organic molecules, such as glutamate. As a part of volume compensatory mechanisms, release of glutamate from astrocytes above physiological level has activating effect on the neurons. Glutamate binding to its receptors leads to opening of ion channels on neuronal membranes and causes their depolarization. The constant influx of Ca^{2+} mainly results in cellular damage and in the cell death eventually. The whole process is known under the term excitotoxicity (Mongin 2016).

Metabolism of glutamate is dependent on specialized transporters in astrocytic membranes, which have much higher affinity and transporting capacity than analogous transporters on neuronal membranes of glutamatergic synapses (Hertz et al. 1999). In astrocytes, the transport of organic molecules through plasmatic membrane is driven by osmotic gradients. Energy source is a difference between concentrations of ions and molecules in ECS and inside the astrocytes. One molecule of glutamate is cotransported with three ions of Na^+ (or two Na^+ and one H^+) and is exchanged with one K^+ and one OH^- (or one HCO_3^-) (Pellerin & Magistretti 1994). But the creation of ionic imbalance is not the only effect of glutamate uptake. Glutamate itself is a very good fuel for cell metabolism, almost as valuable as glucose (Hertz et al. 1999). The transport of glutamate stimulates uptake of glucose and its phosphorylation in astrocytes. It was shown, that this process is strictly stereospecific and only L-Glutamate has effect on glucose utilization (Pellerin & Magistretti 1994; Drejer et al. 1982). As was mentioned earlier, glutamate uptake is Na^+ dependent. If the $[Na^+]_e$ decreases below physiological level, the uptake of glutamate slows down and the same happens to stimulated glucose transport (Pellerin & Magistretti 1994).

5 *In vivo* experiments

Most of the above mentioned data and experiments were accomplished *in vitro*. In this type of experiments, there is a great chance that the data are distorted. The experiments are performed on astrocytes only, so there is a possibility that *in vivo* the cells would react differently to stimulation or inhibition. This is especially obvious in the experiments on brain tissue, because *in vivo* the cells behave as syncytium compared to isolated cells. But breeding of experimental animals is expensive and requires specialized equipment and experienced people. It has also much higher requirements on people's time. On the other hand, results gained in *in vivo* experiments are closer to the real functioning of human brain. Rodents are the most common animals used for brain edema experimental modeling.

The changes in ion homeostasis are examined using channel activators and inhibitors. In the studies using cultured cells, usually functioning of only one protein is tested. Its activity is observed in different conditions using brain edema simulations. That can be achieved with experimental models of ischemia (oxygen – glucose deprivation; MCAO) or with a TBI model.

There is evidence that individual channels affect cellular volume, but only a few of these studies were made using animals. These experiments have shown, that the situation in brain tissue is more complicated than it was thought. For example the channels affect each other – gene expression, localization and activity. There is a relationship between NKCC1 cotransporter and TRPV4 channel as well as between NKCC1 and VRACs (Lu et al. 2016; Mongin 2016). All the channels showed upregulation during development of cerebral edema, which affects cellular swelling. It was found that inhibition of NKCC1 results in reduction of TRPV4 upregulation. But this relationship seems to be just one-way, since the blockage of TRPV4 channel had no effect on the NKCC1 expression. This study suggests that NKCC1 is essential for TRPV4 activation (Lu et al. 2016).

Except from NKCC1, TRPV4 channels create complexes and influence functioning of other channels. For the brain edema formation, a complex of TRPV4, Kir4.1 and AQP4 channels is essential. As was mentioned in previous chapter, the localizations of these channels are overlapping (Jo et al. 2015). Moreover, that for the functioning of AQP4, TRPV4 channels are not required, but the activation of TRPV4 increases cellular swelling in both wild type and AQP4-null mice (Jo et al. 2015).

The problematic of channels and transporters and their role in brain edema is also complicated by the fact that not every astrocyte expresses all the channels and their localization is suspected to be a part of complicated mechanisms of ionic homeostasis and BBB. The Kir4.1 channel is expressed mainly by a subpopulation of astrocytes surrounding blood vessels and neuronal synapses (Higashi et al. 2001). The Kir4.1-null mice showed decreased glutamate uptake and reduced K^+ currents. At low $[K^+]_e$, the Kir4.1 channels became permeable for Ca^{2+} and thus contribute to an incrase in $[Ca^{2+}]_i$ (Seifert et al. 2009). Also TRPV4 channels were found to be abundant in the astrocytes surrounding blood vessels, probably being another part of BBB and they also increase $[Ca^{2+}]_i$ (Benfenati et al. 2007). Calcium as an intracellular signaling molecule causes spreading of pathological conditions, as was described in the connection with TRPV4 channels.

There are still only a few articles describing the role of ion channels in developing brain edema *in vivo.* The central role is usually ascribed to the AQP4 channels, since they are the main astrocytic water passage. But as was described in the part about AQPs, their role in regulation of cellular volume is only passive. Water is driven by the ionic concentration, so the researchers should be more focused on the functioning of ionic channels and transporters. The roles of other channels and their connection to AQP4 - and to each other - still need to be examined thoroughly, because the mechanisms of RVD and other volume regulations were mainly examined in astrocytes – there is a lack of data describing behavior of other cell types and their influence on astrocytes. There is also not enough evidence for the function of specific channel inhibitors - and their potential role as anti-edema drugs – in mouse models, because *in vitro* studies are definitely not sufficient and the mechanisms described in this thesis would probably not be working with the same efficiency on laboratory animals.

Moreover, if a pharmacological treatment successfully works against edema in rodent models, it is not the case in humans. This discrepancy might be caused by underestimated differences in between the rodent and human brain. As mentioned earlier, there are significant differences in astrocyte morphology, which might be accompanied by differences also in their function. For example, water movements inside the brain probably differ from those described in this thesis. New experiments have shown large differences in AQP4 expression and localization in human astrocytes compared to rodent astrocytes (Eidsvaag et al. 2017).This suggests that water clearance mechanisms, which are described on rodent models, are actually not the same in human brains. There is also not evidence about swelling of other cell types in the human brain – neurons in the first place.

6 Treatment of cerebral edema

Previous chapter described our lack of understanding for the processes leading to cellular swelling and brain edema, mainly because of the complexity of brain tissue. Since the brain edema is one of the leading death causes around the world, researchers and physicians are still trying to find the best way to treat patients suffering from this pathology. There is no medication, since we still do not know all the principals behind cerebral edema.

In the first chapter, brain edema was described as a water content increase and thus a volume change. The most dangerous part – along with apoptotic processes – is a risk of tissue compression, even in the parts of brain not directly adjoining the primary lesion. This can cause neuronal damage and lead to death of patients. The diagnostic of a developing brain edema is difficult because usually the edema causes loss of consciousness due to raised intracranial pressure (Rabinstein 2006; Raslan & Bhardwaj 2007). The edema however can be recognized using imaging techniques, such as MRI or computer tomography (CT). On MRI scans, even the type of edema is recognizable, but not in every case. The treatment of a spreading edema depends on its cause – whether the patient suffers from a stroke, a liver failure or for example TBI. The patients should be ventilated and usually sedated to prevent pain, anxiety and agitation, which increases already high intracranial pressure (Rabinstein 2006; Raslan & Bhardwaj 2007). The high pressures onside the brain parenchyma can be reduced using decompressive craniectomy. It is a surgical intervention of perforating the skull.

Through the history there was an attempt to solve developing brain edema using osmotherapy. A hypertonic solution was applied. Researchers hoped, that changed osmotic pressures would be a driving force for water to leave the injured brain tissue and so the impact of pathological conditions would be lessen. As the hypertonic solution, concentrated human plasma proteins or glycerol were used. And glycerol is used ever since by some physicians, because of tradition. But there are still ongoing studies, focused on the use of hypertonic solutions and its best composition (Raslan & Bhardwaj 2007).

Another orientation of current research is, of course, invention of best anti-edema drugs to decrease neurological damage. For cytotoxic cellular swelling, the main targets of the medication seem to be AQP4 and ion channels and transporters. There are already molecules known do decrease their activity. Some of those are used in patients, but most of them are used for animal models only. But the progression in the cerebral edema issue is expected in the future (Michinaga & Koyama 2015).

7 Conclusion

In this thesis, the recent findings about mechanisms involved in the brain edema formation were presented. In previous chapters the types of edema were described and compared. Cytotoxic edema is characteristic by cellular swelling – increasing astrocytic water content and volume changes. Cerebral edema occurs in connection with numerous brain pathologies, such as stroke or TBI.

There are still many questions about the problematic of brain edema - mainly, whether the described processes would work *in vivo*. Researchers are trying to find the best way to decrease spreading of an edema and to prevent neuronal damage. But the complexity of brain tissue forces them to take all the cell types and their connections in consideration. There is still a lot of work to be done before the mechanisms of cytotoxic cellular swelling and spreading of cerebral edema will be fully understood. Studying mechanisms of brain edema formation in transgenic animals using advanced imaging techniques is a possible direction of future research works.

8 Abbreviations

ATP	Adenosine triphosphate	LRRC8, LRRC8A	Subunits of VRAC channels
AQP	Aquaporin	MCAO	Middle cerebral artery occlusion
BBB	Blood brain barrier	MRI	Magnetic resonance imaging
Ca^{2+}	Calcium cation	Na^{+}	Sodium cation
Cl^{-}	Chloride anion	NKCC	Sodium/potassium/chloride cotransporter
CNS	Central nervous system	OH^{-}	Hydroxide anion
CT	Computer tomography	RVD	Regulatory volume decrease
ECS	Extracellular space	RVI	Regulatory volume increase
GFP	Green fluorescent protein	siRNA	Small interfering RNA
HCO_3^{-}	Hydrogen carbonate anion	TBI	Traumatic brain injury
HeLa cells	An immortal human cell line used for scientific research	TRP	Transient receptor potential
HEK cells	Human Embryonic Kidney cells	TRPV	Transient receptor potential vanilloid
ICS	Intracellular space	VRAC	Volume regulated anion channel
K_{2P}	Two pore domain potassium channel	WT	Wild type
K^{+}	Potassium cation	$[X^{+}]_i$	Intracellular ionic concentration
Kir	Inwardly rectifying potassium channel	$[X^{+}]_e$	Extracellular ionic concentration
K_V	Voltage gated potassium channel		

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